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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)
	) Group Art Unit: 1655
Hajime Matsuzaki et al.	)
	) Examiner: F. Lu
Serial No. 09/099,301	) Box AF
	)
Filing Date: June 18, 1998	) Docket No. 03848.74891

For: **METHODS AND COMPOSITIONS FOR MULTIPLEX AMPLIFICATION OF NUCLEIC ACIDS**

DECLARATION OF HAJIME MATSUZAKI

I, Hajime Matsuzaki, hereby declare:

1. I am an inventor of the application referenced above.

2. I have reviewed and am familiar with Diamandis, et. al., U.S. Patent 5,552,283, "Method, Reagents and Kit For Diagnosis and Targeted Screening For P53 Mutations" by Diamandis, issued September 3, 1996.

3. I am aware that the United States Patent and Trademark Office has taken the position that Diamandis, et. al. inherently teaches the ratio of primers recited in claim 15 of our application, referenced above, *i.e.*, $C_A=C_L(L_A \div L_L)^2$.

4. Diamandis teaches the use of 150 ng of each primer in a multiplex polymerase chain reaction (column 11, line 46). This is inconsistent with the concentration of primers required by the formula recited in claim 15.

5. I have calculated the molar ratio of primers taught by Diamandis. It does not conform to the ratio required by our claim 15. Multiplex pool A of the Diamandis reference employs the following molar ratio of primers, where the longest exon is set at one: 1.07 (exon 1): 1.14 (exon 3): 0.96 (exon 4): 1.13 (exon 5): 1.13 (exon 6): 1.07 (exon 9): 1.00 (exon 10): 1.08 (exon 11). In contrast, using the calculation recited in claim 15, the molar ratio for each of the primer sets would be, where the longest exon is set to one: 0.72 (exon 1): 0.17 (exon 3): 0.96

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(exon 4): 0.47 (exon 5): 0.40 (exon 6): 0.29 (exon 9): 1.00 (exon 10): 0.43(exon 11). The underlying facts used to obtain these ratios are presented in the table. Similarly, the primer concentrations taught for amplification of multiplex pool B in the Diamandis reference are inconsistent with those recited by our formula. The molar primer ratio used by the Diamandis reference is 1.17 (exon 2): 1.00 (exon 8), whereas claim 15 of our application requires a molar ratio of primers of 0.67 (exon 2): 1.00 (exon 8). Again, the concentration of primers used by Diamandis is inconsistent with that required in our application. Therefore, the Diamandis reference fails to teach a molar ratio of primers that conforms to that recited in the claims of the subject application.

6. The following table provides the underlying facts used to determine the molar ratio of primers used in each multiplex polymerase chain reaction by the method taught by Diamandis, and the calculation recited in claim 15

For multiplex pool A:

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Amplicon Number	Exon Size (bp)	Primers Used to Amplify Each Exon	Molecular Weight of Each Primer	Concentration of each Primer when 150 ng is used in a 50 µl reaction (nM) (Diamandis)	Concentration of Primers for Amplification of each Exon using 150 ng each primer in a 50 µl reaction (nM) (Diamandis)	Molar Ratio of Primers Based on use of 150 ng each primer in a 50 µl reaction (Diamandis)	Molar ratio determined by formula recited in claim 15: $C_A = C_L(L_A \div L_L)^2$
1	331	5'-CGGATTACTTGCCCTTACTTGTCA-3'	7270.7	412.6	940.3	1.07	0.72
		5'-CCCCAGCCCCAGCGATTTT-3'	5685.7	527.6			
3	162	5'-CATGGGACTGACTTTCTGCT-3'	6100.0	491.8	1005.5	1.14	0.17
		5'-GGACGGCAACGCCGACTGT-3'	5839.8	513.7			
4	382	5'-CTGGTCCTCTGACTGCTCTTTTCA-3'	7237.7	414.5	848.8	0.96	0.96
		5'-AAAGAAATGCAGGGGATACGG-3'	6907.5	434.3			
5	268	5'-TGTTCACTTGTGCCCTGACT-3'	6050.9	495.8	995.3	1.13	0.47
		5'-CAGCCCTGTCGTCTCTCCAG-3'	6005.9	499.5			
6	247	5'-CTGGGGCTGGAGAGACGACA-3'	6233.1	481.3	995.6	1.13	0.40
		5'-GGAGGGCCACTGACAACCA-3'	5832.8	514.3			
9	209	5'-GCGGTGGAGGAGACCAAGG-3'	5968.9	502.6	944.4	1.07	0.29
		5'-AACGGCATTTTGAGTGTTAGAC-3'	6790.5	441.8			
10	390	5'-TGATCCGTCATAAAGTCAAACAA-3'	7025.6	427.0	882.2	1.00	1.00
		5'-GTGGAGGCAAGAATGTGGTTA-3'	6591.3	455.1			
11	256	5'-GGCACAGACCCTCTCACTCAT-3'	6312.1	475.3	949.0	1.08	0.43
		5'-TGCTTCTGACGCACACCTATT-3'	6333.1	473.7			

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For multiplex pool B:

Amplicon Number	Exon Size (bp)	Primers Used to Amplify Each Exon	Molecular Weight of Each Primer	Concentration of each Primer when 150 ng is used in a 50 µl reaction (nM) (Diamandis)	Concentration of Primers for Amplification of each Exon using 150 ng each primer in a 50 µl reaction (nM) (Diamandis)	Molar Ratio of Primers Based on use of 150 ng each primer in a 50 µl reaction (Diamandis)	Molar ratio determined by formula recited in claim 15: $C_A = C_L(L_A \div L_L)^2$
2	261	5'-ACCCAGGGTTGGAAGCGTCT-3'	6159.0	487.1	929.1	1.17	0.67
		5'-GACAAGAGCAGAAAGTCAGTCC-3'	6787.5	442.0			
8	320	5'-GACAAGGGTGGTTGGGAGTAGATG-3'	7579.0	395.8	794.1	1.00	1.00
		5'-GCAAGGAAAGGTGATAAAAGTGAA-3'	7533.0	398.2			

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States; and further that false statements made willfully may jeopardize the validity of any patent issuing on an application in which the false statements were made.

6/20/01
Date

Hajime Matsuzaki